

# THERMAL TOLERANCE AND RESISTANCE OF THE NORTHERN ANCHOVY, *ENGRAULIS MORDAX*

GARY D. BREWER<sup>1</sup>

## ABSTRACT

An experimental, flow-through seawater system, constructed to maintain juvenile and adult northern anchovy, *Engraulis mordax*, and rear embryos and larvae through yolk-sac absorption under controlled temperature and photoperiod regimes, was used to determine aspects of thermal tolerance, resistance, rates of acclimation, and some effects of temperature on the development and growth of the anchovy.

Thermal tolerance was determined for juvenile and adult fish, acclimated to six constant temperatures between 8° and 28°C. Thermal resistance (minutes until death for fish exposed to a lethal temperature) was independent of photoperiod and fish size; however, females proved more resistant than males, and resistance decreased at night. Acclimation (as measured by resistance) from 12° to 20°C was nearly complete after 2-day exposure to the higher temperature; acclimation from 20° to 12°C was nearly complete after 5-day exposure to the lower temperature. Fish subjected to fluctuating water temperatures between 12° and 20°C proved less resistant to cold than a 12°C (constant) acclimated group and less resistant to heat than a 20°C (constant) acclimated group.

Thermal tolerance was determined for larvae in the yolk-sac stage, acclimated to four constant temperatures between 12° and 24°C. Although hatching occurred at temperatures as high as 29.5°C and as low as 8.5°C, the percentage of normally developed larvae equaled that of controls (incubated at 16°C) only between temperatures of 27.0° and 11.5°C. Embryos in the blastodisc stage proved most sensitive to acute temperature increases when compared to embryos in the blastopore closure stage and larvae in the yolk-sac stage. These same three stages proved insensitive to acute temperature decreases to 0.5°C for 60-min exposure periods.

Temperature is discussed in relation to anchovy distribution and survival under natural and artificially created thermal conditions.

Research on the effects of temperature on aquatic organisms has been given impetus in recent years as numerous lakes and streams are considered potential heat reservoirs by electric power generating plants and other industrial concerns. As the demands for water as a heat transfer medium continue to increase dramatically, more attention will be turned to the marine environment for large volumes of water and surface areas necessary for the dissipation of excess heat (Naylor 1965; de Sylva 1969; Tarzwell 1972). Unchecked thermal loading of freshwater and near-shore marine ecosystems will inevitably pose a serious threat to the homeostasis and well-being of aquatic communities unless realistic guidelines are established and enforced. Such guidelines must be based on knowledge of how aquatic organisms respond to both acute and chronic temperature changes.

This study details aspects of thermal tolerance and resistance (as defined by Fry 1971) on the

embryo, larval, juvenile, and adult stages of the northern anchovy, *Engraulis mordax* Girard. The study was prompted by the proposed discharge of thermal effluent into the Los Angeles-Long Beach Harbor. The biology and fishery of the northern anchovy in the Los Angeles-Long Beach Harbor were described by Brewer (1975a).

The general biology of the northern anchovy has been summarized by Baxter (1967), Messersmith et al. (1969), the California Department of Fish and Game (1971), and Brewer (1975a). A dramatic increase in abundance of *E. mordax* during the past 20 yr (Ahlstrom 1967; Smith 1972) has prompted an intense interest in the biology and fishery potential of this clupeoid. The California Department of Fish and Game (1971:48) considered the anchovy "... the most abundant species with immediate harvest potential in the California Current system."

## MATERIALS AND METHODS

Experiments were conducted in a small, tem-

<sup>1</sup>Allan Hancock Foundation, University of Southern California, University Park, Los Angeles, CA 90007.

perature and photoperiod controlled, flow-through seawater system. The system delivered filtered, ultraviolet-sterilized seawater from the Los Angeles-Long Beach Harbor to five round, 950-liter, fiber glass aquaria (1.5 m in diameter, 0.6 m high) and a single 400-liter rectangular water table, all housed in a light-tight aluminum cargo container (Figure 1). An exchange rate of 2-6 liters/min was maintained in each aquarium, with overflow drainage provided by standpipes. Wastewater was not recirculated. Temperatures were maintained within  $\pm 0.5^{\circ}\text{C}$ .

Above each aquarium were two incandescent light bulbs controlled by separate dimmer controls and regulated by a 7-day timer to simulate photoperiods. The "day" bulb provided 700 lx and the "night" bulb provided 16 lx to the surface of each aquarium. Oxygen was maintained at or near saturation levels in all acclimation and test tanks by splashing incoming water at the surface and by bubbling air stones in the aquaria. Salinity varied between 31.4 and 33.8‰ (mean 33.1‰) during the study period.

### Juveniles and Adults

Juvenile and adult *E. mordax* were obtained from a live-bait dealer. The initial transfer from the bait boat to the 950-liter acclimation tanks caused 20-30% mortality during the first 2-3 days of confinement. Within 2-4 days, healthy fish began to feed and were offered a daily ration, equivalent to approximately 4% of the fish's wet weight,

of Trout Chow.<sup>2</sup> This ration was supplemented with chopped anchovy, chopped squid, brine shrimp, or wild plankton equal to approximately 1% of the fish's wet weight. Adjusted fish ate voraciously and mortality became insignificant in acclimation tanks within 1 wk. Acclimation tanks were stocked with between 3 and 7 kg of anchovy. The food ration was withheld for a period of 24 h prior to all thermal tests on juvenile and adult fish.

### Ninety-six Hour Tolerance

Standardized techniques for the determination of lethal temperatures (Fry et al. 1942; Brett 1944; Fry 1947) call for a series of experiments in which the animals are acclimated to several different constant temperatures. Acclimated fish are then abruptly transferred to test aquaria previously equilibrated to various high and low temperature extremes. Mortality is monitored and recorded.

This procedure extends the concept of lethal temperatures from two extreme end points, to a family of upper and lower (incipient) lethal levels. The ultimate upper and lower lethal temperatures, which circumscribe the extreme tolerance limits, may be determined by graphic extrapolation—that is, by drawing a line through those high and low test temperatures that proved lethal to 50% of the test animals for each acclimation temperature. The extrapolated line will then

<sup>2</sup>Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

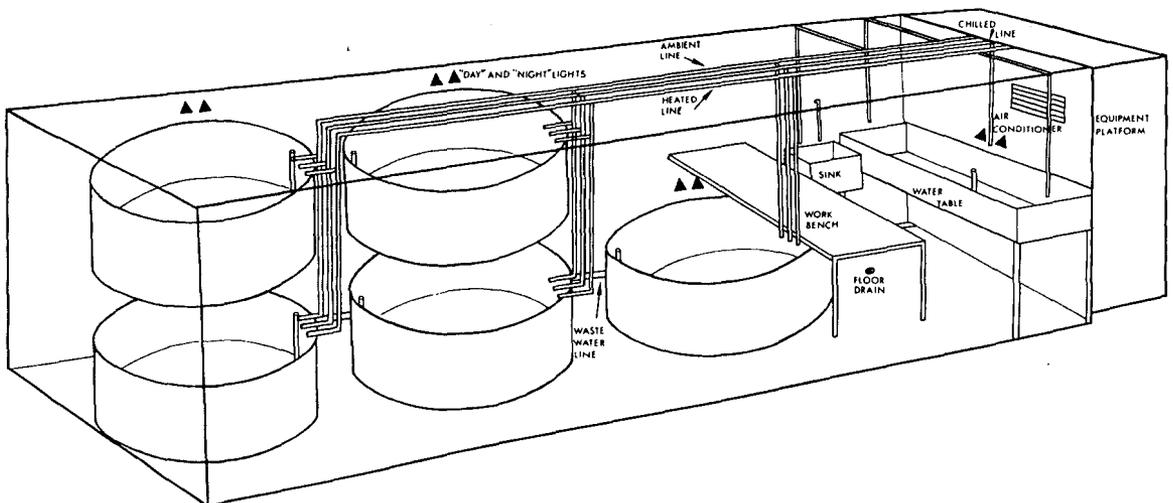


FIGURE 1.—Diagram of the flow-through seawater system used for experiments on *Engraulis mordax*.

intersect a diagonal at the upper and lower extremes, which represents those points where the lethal temperatures equal the acclimation temperatures. Fish cannot be acclimated to temperatures above or below these experimentally determined ultimate upper and lower lethal temperatures, respectively.

Juvenile and adult *E. mordax* between 45 and 139 mm SL (standard length) were held for a minimum of 3 wk at constant temperatures of 8°, 12°, 16°, 20°, 24°, and 28°C and under a light cycle of 12 h light and 12 h dark. Unless otherwise noted, the term "acclimated fish" designates *E. mordax* held under such conditions. "Juvenile" refers to metamorphosed fish less than 100 mm SL, while "adult" refers to fish over 100 mm.

The fish's susceptibility to mechanical damage increased at high and low acclimation temperatures. Therefore, each acclimation temperature-test series was accompanied by a series of strict control transfers, and the observed mortalities for each series were adjusted separately, based on the respective control mortalities. Fish were considered dead and were removed when all swimming movements ceased. Ninety-six hour LD<sub>50</sub> (mean lethal dose) temperatures (i.e., incipient lethal levels) were estimated from regression lines plotted on probit paper (Sokal and Rohlf 1969). Experiments were conducted between February 1973 and November 1974 and included all seasons. About 20 fish were used for each test.

#### Resistance as a Function of Size, Sex, Time, and Photoperiod

To test the potential influence of photoperiod, sex, size, and diel effects, anchovies were acclimated to 20°C, tested by direct transfer to 30°C, and the time to death (resistance time) determined. As the fish died, they were removed from the test aquaria, measured, and adult fish were sexed. Identical tests were conducted in the morning (0900 h) and in the evening (2100 h). Tests were also conducted after fish had been held under a short-day photoperiod (8 h light) and a long-day photoperiod (16 h light) for periods of 3 wk each. All thermal resistance tests were run during the summer and fall.

#### Rates of Thermal Acclimation

Juvenile and adult anchovy acclimated to 12°C were subjected to an 8°C temperature change over

a 24-h period to 20°C, and then tested for resistance to 30°C on the same day and after 1-, 2-, and 4-day exposure to the 20°C temperature. Moreover, fish acclimated to 20°C were subjected to a temperature decrease to 12°C over a 24-h period, and then tested for resistance to 6°C on the same day and after 2-, 5-, and 9-day exposure to the 12°C temperature. As the fish become acclimated to the new higher or lower temperature, one would expect the mean resistance times for these fish to approach and eventually equal the mean resistance times (e.g., reach a steady-state) of fish acclimated to 20° and 12°C and tested at 30° and 6°C, respectively (controls).

#### Effects of Cycled Temperatures on Resistance

In view of the observations by Mais (1974) that *E. mordax* may undergo diel vertical migrations and consequently experience fluctuating temperatures, I examined the relative thermal resistance of anchovies subjected to regular changes in temperature from 12° to 20°C over 48-h intervals. Fish acclimated to 20°C were gradually subjected to decreasing temperatures to 12°C over 24 h and then back to 20°C over the next 24 h. The cycle was repeated for 25 days, at which time a sample of fish which had just reached 20°C was tested for resistance to 30°C. The following morning, as the remaining fish reached 12°C, a sample was tested for resistance to 6°C.

#### Embryos and Larvae

*Engraulis mordax* eggs, caught in plankton tows in or near the Los Angeles-Long Beach Harbor throughout the year, were utilized for experiments on embryos and larvae. Water temperatures, at time of capture, varied between 13° and 18°C. In the laboratory, eggs in the blastodisc stage were placed into 2-liter glass jars and maintained at 12°, 16°, 20°, or 24°C until transferred to incubation or test vessels which consisted of 250-ml jars containing 60 ml of seawater. Not more than five eggs or larvae were tested per jar.

#### Twenty-four Hour Tolerance

Larvae in the yolk-sac stage were tested within 1 day after hatching at each acclimation temperature. Larvae were pipetted from each acclimation temperature directly into test vessels ranging

from 6° to 32°C. Mortality was recorded after 24 h. Incipient lethal levels for each acclimation level were estimated as described for the juveniles and adults.

### Hatching and Developmental Temperature Limits

Eggs in the blastodisc stage were transferred to a series of incubation vessels, after which the temperatures were gradually raised or lowered from the ambient level of 16°C over a period of 60 min in order to avoid possible shock effects to the developing embryos. Incubation temperatures were then held constant ( $\pm 0.5^\circ\text{C}$ ) between 6° and 12°C, and 26° and 31°C at 0.5°C intervals. A 16°C temperature was used as a control. Development was considered normal only if the larvae were free of obvious deformities (e.g., spinal curvatures) until pigmented eyes and functional jaws were evident, and death had occurred only after yolk reserves were exhausted.

### Resistance to Acute Temperature Changes

Embryos in the blastodisc stage (ca. 12-14 h after fertilization at 16°C) and in the blastopore closure stage (ca. 36-38 h after fertilization), and larvae in the yolk-sac stage (within 24 h after hatching) were subjected to temperature shocks for periods of 1, 3, 5, and 60 min. Embryos and larvae were pipetted from incubation vessels maintained at 16°C directly into water at high and low temperature extremes. After the exposure period, the embryos and larvae were returned directly to the incubation vessels at 16°C where they remained for 48 h after hatching. Mortality and developmental abnormalities were recorded.

This procedure was an attempt to simulate what the embryos and larvae might actually experience if entrained by intake pipes of electrical generating plants or LNG (Liquified Natural Gas) vaporization plants (or either thermal plums), subjected to rapid temperature increases and decreases in the heat exchange systems, and subsequently flushed back into the natural environmental temperatures at the outfall.

### Development and Growth

Experiments were designed to determine the temperatures required for optimal growth of an-

chovy larvae. The tests were confined to that period of larval life between hatching and starvation following exhaustion of all stored yolk reserves. No food was offered.

Eggs in the blastodisc stage were reared through hatching in a series of constant temperature baths between 10° and 26°C. On the day of hatching and each subsequent day, approximately 10 larvae were sacrificed from each rearing temperature and measured from the tip of the snout to the end of the notochord with an ocular micrometer to the nearest 0.05 mm. This procedure was continued until all larvae at each rearing temperature died of starvation.

## RESULTS

### Juveniles and Adults

#### Ninety-six Hour Tolerance

Experiments on juvenile and adult tolerance encompassed 117 separate 96-h tests and 2,400 fish. Control survival ranged from lows of 81.3 and 87.9% at 8° and 28°C acclimation temperatures, respectively, to 98.3% at the 16°C acclimation temperature.

Figure 2 graphically depicts the lethal temperature relations, with adjusted percent mortality plotted against test temperatures for acclimation levels of 8° and 28°C. Adjusted upper and lower LD<sub>50</sub> temperatures were plotted against acclimation temperatures in Figure 3 and a thermal tolerance polygon constructed (Fry 1947). Ultimate upper and lower lethal temperatures are estimated by extrapolation (line fitted by eye) to be

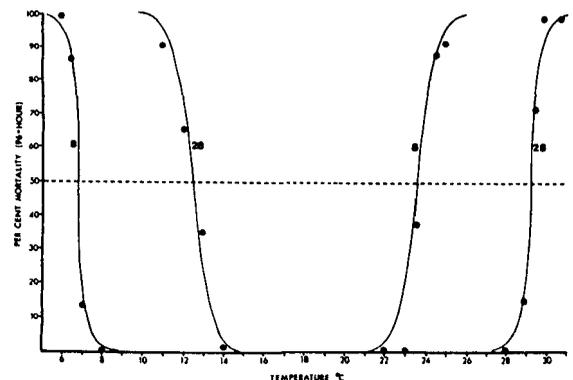


FIGURE 2.—Effects of acclimation temperatures of 8° and 28°C on the upper and lower lethal temperatures of *Engraulis mordax* juveniles and adults (original data in Brewer 1975b).

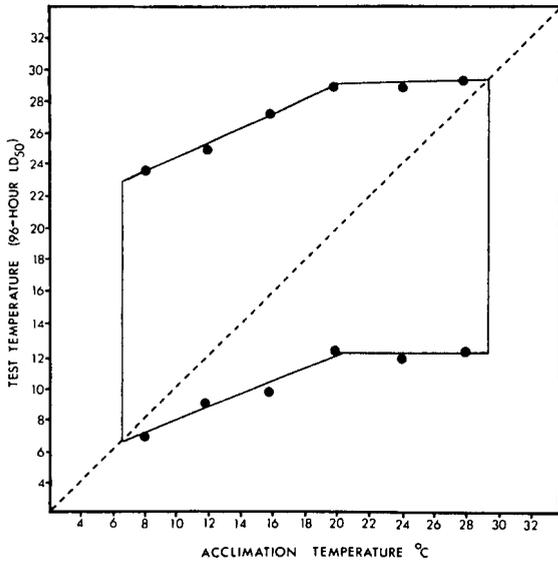


FIGURE 3.—Thermal tolerance polygon for *Engraulis mordax* juveniles and adults. Those points where the extrapolated LD<sub>50</sub> levels intersect the diagonal represent the extreme (ultimate) tolerance limits and correspond to 6.5° and 29.5°C.

29.5° and 6.5°C, respectively. These temperatures represent the maximum tolerance range of *E. mordax* juveniles and adults sampled from southern California and maintained under laboratory conditions as described. Anchovy cannot be acclimated to temperatures beyond these extremes. Attempts were made to slowly acclimate fish to 29.5° and 6.5°C, but they proved futile.

Figure 4 shows the resistance times to median mortality of juvenile and adult *E. mordax*, acclimated to 8°, 16°, and 28°C, upon exposure to temperatures beyond incipient lethal levels. These curves were derived by plotting cumulative mortality as percentages against exposure time to estimate the time to LD<sub>50</sub> for each test temperature.

**Resistance as a Function of Size, Sex, Time, and Photoperiod**

Results of experiments on thermal resistance to 30°C in relation to size, sex, and potential diel and photoperiod effects are summarized in Table 1. Analysis of variance (one-way classification) showed that resistance times to lethal temperatures of 30°C were not significantly different ( $P > 0.05$ ) for fishes of different sizes (<79 mm; 80-99 mm; >100 mm) or for fishes maintained under different photoperiods (8, 12, and 16 h

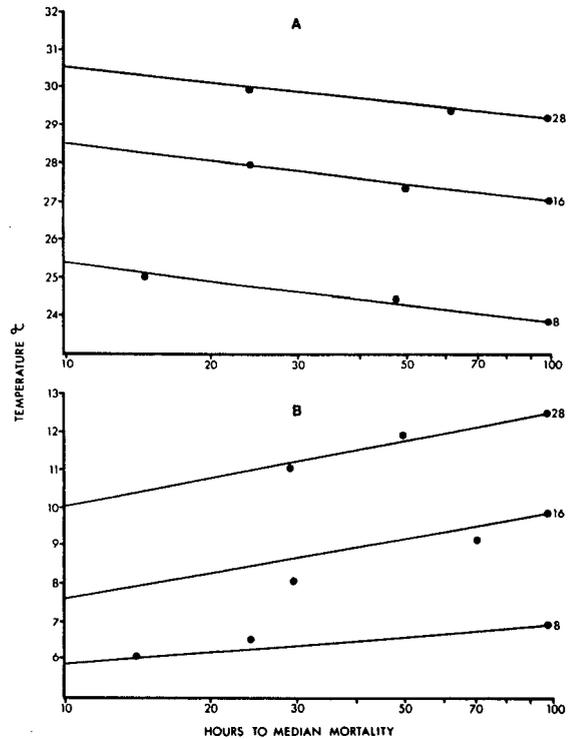


FIGURE 4.—Resistance times to median mortality of juvenile and adult *Engraulis mordax* exposed to high (A) and low (B) lethal temperatures when acclimated to 8°, 16°, and 28°C.

TABLE 1.—Comparison of resistance times (minutes until death) for juvenile and adult *Engraulis mordax* acclimated to 20°C and immediately transferred to aquaria at 30°C.

Item	N	Range (min)	Mean (min)	SD	SE
Length (mm):					
<79	11	49-285	133.9	74.9	22.6
80-99	22	41-302	149.4	62.3	13.3
>100	12	37-343	141.3	102.9	29.7
Sex:					
Male	20	6-118	40.9	33.1	7.4
Female	36	4-401	116.8	113.6	18.9
Time of test:					
Morning	45	31-343	143.5	76.3	11.4
Evening	38	8-244	72.6	66.7	10.8
Photoperiod (hours of light):					
8	14	6-401	154.5	127.0	34.0
12	34	37-302	141.1	67.6	11.6
16	11	31-343	150.8	102.3	30.9

light). These results should be verified with larger sample sizes. Resistance times showed highly significant differences ( $P < 0.01$ ) for males compared with females, and for tests conducted in the morning as compared with those conducted at night. Females proved more resistant than males, and animals tested in the morning showed greater resistance than those tested in the evening.

## Rates of Thermal Acclimation

Results suggest that acclimation from 12° to 20°C nears completion within 2 days of exposure to the higher temperature. Mean resistance times for day 2 and day 5 samples exceeded the mean resistance time for the control sample. However, analysis of variance shows that the variation between day 2, day 5, and control samples is not significant ( $P > 0.05$ ). The relatively high resistance of some fish in the day 2 and day 5 samples, which exceeded the resistance of control fish, may be due to slight temperature variations ( $\pm 0.2^\circ\text{C}$ ) in the test aquaria, or possibly to "physiological overshoots" to the acclimation process (Prosser 1973). Figure 5 shows the progress toward acclimation with continued exposure to the higher temperature. Most noticeable is the change in shape of the resistance curves with acclimation. Nonacclimated fish succumb to the lethal 30°C temperature quickly, probably as a result of "shock effects" (Scott 1964; Tyler 1966; Allen and Strawn 1971). Acclimation to the higher temperature diminishes the shock effects. Apparently, acclimated fish die from secondary causes termed "direct effects" by Fry (1971). The physiological basis of the shock and direct effects is not clear. Acclimation from warm to cool water (20° to 12°C) appears to be nearly complete by day 5 (Figure 6). As acclimation progresses and resistance to low temperatures is increased, death rate becomes in-

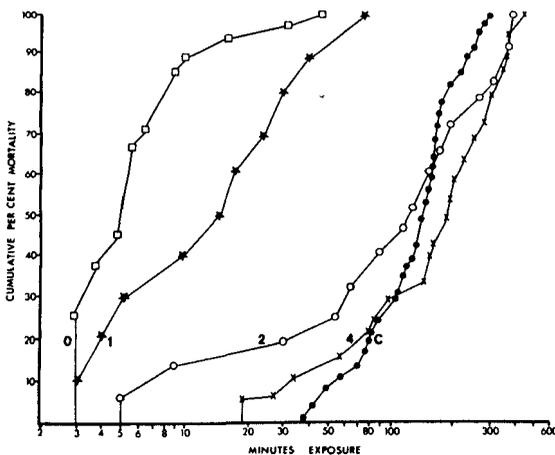


FIGURE 5.—Cumulative percent mortality of *Engraulis mordax* juveniles and adults as a function of exposure to 30°C. The response of a 20°C acclimated control group (C) is compared with that of a 12°C acclimated group after 0-, 1-, 2-, and 4-day exposures to 20°C (original data in Brewer 1975b).

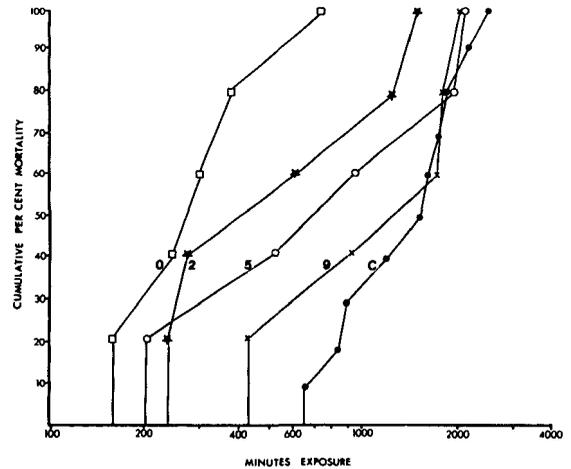


FIGURE 6.—Cumulative percent mortality of *Engraulis mordax* juveniles and adults as a function of exposure to 6°C. The response of a 12°C acclimated control group (C) is compared with that of a 20°C acclimated group after 0-, 2-, 5-, and 9-day exposures to 12°C (original data in Brewer 1975b).

creasingly regular; the graphs approach a straight line and the effects of the initial cold shock are largely eliminated. Because these tests used small sample sizes, statistical differences cannot be demonstrated.

## Effects of Cycled Temperatures on Resistance

Results are summarized in Table 2. Mean resistance times to 6° and 30°C for fish subjected to

TABLE 2.—Resistance times (minutes until death) of juvenile and adult *Engraulis mordax* to 6° and 30°C after being subjected to temperature fluctuations between 12° and 20°C on a 48-h cycle for a period of 25 days.

Test temp	Group	N	Range (min)	Mean (min)	SD	SE
6°C	12°C acclimated	10	643-2,490	1,419.0	589.0	186.3
	20° to 12°C	10	117-1,111	410.6	374.5	118.4
30°C	20°C acclimated	34	37-302	141.1	67.6	11.6
	12° to 20°C	10	6-68	28.0	22.1	7.0

periodic changes in temperature between 12° and 20°C were well below the mean resistance times of fish acclimated to a constant 12°C and constant 20°C, respectively. However, the fish have greater high temperature resistance than those acclimated to 12° and greater low temperature resistance than those acclimated to 20°C.

## Embryos and Larvae

### Twenty-four Hour Tolerance

Over 600 larvae were tested in the 24-h tolerance experiments. Generally, 10 or more larvae were tested at each temperature. The percentage (normal) survival for controls ranged from 72.7 at the 12°C acclimation level to 86.7 at 16° and 20°C acclimation temperatures.

Apparently the physiological mechanisms for thermal acclimation are little developed in *E. mordax* larvae in the yolk-sac stage. Figure 7 shows the 24-h lethal temperature relations with percent adjusted mortality plotted against test temperatures for acclimation temperatures of 12° and 24°C. Rearing the yolk-sac larvae in warm and cold water does little to increase or decrease their upper or lower lethal temperatures, respectively. Potential effects of parental acclimation temperatures (Hubbs and Bryan 1974) or the exposure of eggs to acclimation temperatures at the time of fertilization require investigation.

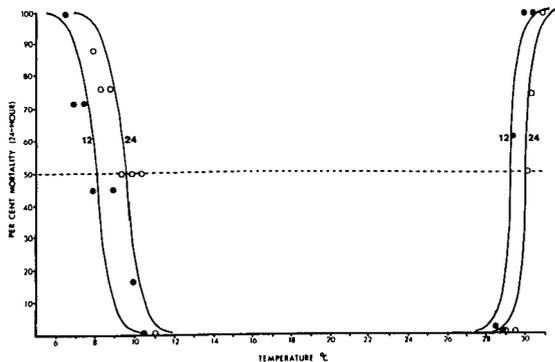


FIGURE 7.—Effects of acclimation temperatures of 12° and 24°C on the upper and lower lethal temperatures of *Engraulis mordax* larvae in the yolk-sac stage (original data in Brewer 1975b).

In Figure 8, adjusted upper and lower LD<sub>50</sub> temperatures are plotted against respective acclimation temperatures in the construction of a thermal tolerance polygon. Ultimate upper and lower lethal temperatures are estimated to be 30.2° and 7.0°C, respectively.

### Hatching and Developmental Temperature Limits

Results of this experiment are given in Table 3. Although hatching was observed at temperatures

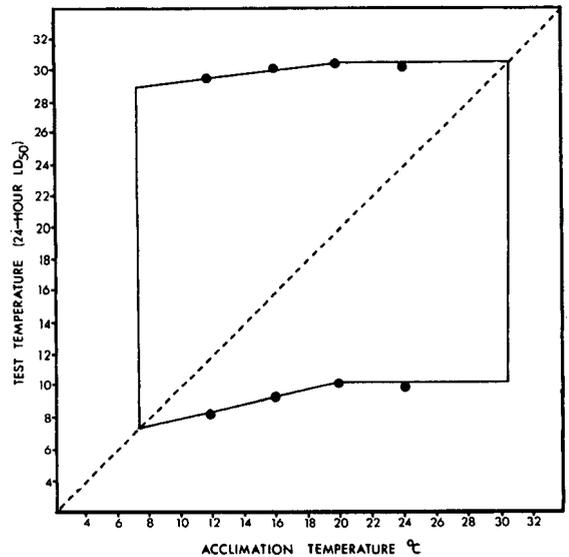


FIGURE 8.—Thermal tolerance polygon for *Engraulis mordax* larvae in the yolk-sac stage. Those points where the extrapolated LD<sub>50</sub> levels intersect the diagonal represent the extreme tolerance limits and correspond to 7.0° and 30.2°C.

TABLE 3.—Effects of temperature on hatching and development of *Engraulis mordax* through yolk-sac absorption and eye pigmentation of larvae. Temperatures were maintained within ±0.5°C of those shown below.

Rearing temp (°C)	N	No. hatching	With normal development		Adjusted survival (%)
			No.	%	
31.0	10	0	0	0	0.0
30.0	10	0	0	0	0.0
29.5	10	3	3	30.0	33.3
29.0	10	3	3	30.0	33.3
28.5	10	8	5	50.0	55.6
28.0	30	27	23	76.7	84.9
27.5	10	9	7	70.0	77.8
27.0	30	28	27	90.0	100.0
26.5	10	8	8	80.0	88.9
26.0	10	9	9	90.0	100.0
16.0	30	29	27	90.0	100.0
7.5	10	0	0	0.0	0.0
8.0	10	0	0	0.0	0.0
8.5	10	2	0	0.0	0.0
9.0	10	3	0	0.0	0.0
9.5	15	3	0	0.0	0.0
10.0	10	6	1	10.0	11.1
10.5	10	5	3	30.0	33.3
11.0	10	8	6	60.0	66.7
11.5	10	9	9	90.0	100.0
12.0	10	10	9	90.0	100.0

<sup>1</sup>Control.

as high as 29.5°C and as low as 8.5°C, 50% (adjusted) normal development occurred between 11.0° and 28.5°C. Only below 27.0°C and above 11.5°C did the percentages of hatching and normal development approach those for the controls.

## Resistance to Acute Temperature Changes

Resistance to high temperatures is surprisingly great for embryos and larvae when exposure is of short duration (Figure 9). Blastodisc stage embryos are least resistant while yolk-sac larvae are most resistant; LD<sub>50</sub> values for the 60-min exposure period for the larvae are within 1.3°C of the extrapolated 24-h tolerance limits determined from Figure 8. *Engraulis mordax* embryos and larvae appear to be insensitive to abrupt temperature decreases down to 0.5°C for short periods (Brewer 1975b).

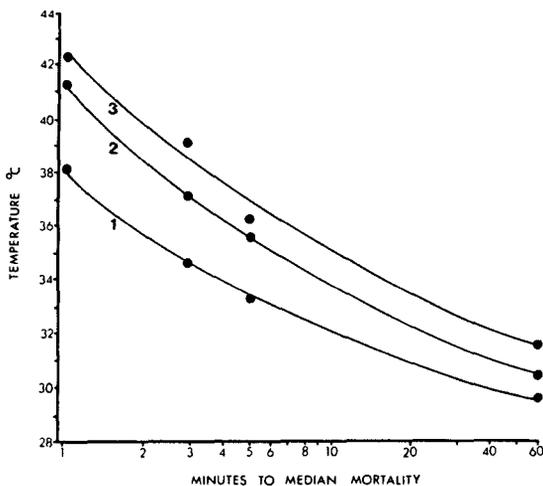


FIGURE 9.—Minutes to median mortality for the blastodisc stage (1), blastopore closure stage (2), and yolk-sac stage (3) subjected to abrupt temperature increases from 16°C (original data in Brewer 1975b).

## Development and Growth

Table 4 summarizes data on the growth of larvae at constant temperatures between 10° and 26°C. The maximum size of larvae attained at any temperature before shrinkage due to starvation was 4.16 mm. This is considerably smaller than the value of 4.8 mm given by Lasker (1964) for *E. mordax* larvae reared under similar conditions. Variability in egg size may be responsible for this discrepancy; egg size of the Argentine anchovy, *E. anchoita*, is known to vary by season and location (de Ciechowski 1973).

The highest mean growth response was obtained for larvae reared at 18°C (3.94 mm). Mean larval lengths less than 3.78 mm were considered

TABLE 4.—Comparison of the maximum size attained by *Engraulis mordax* larvae in the yolk-sac stage before shrinkage due to starvation.

Incubation temp (°C)	N	Range (mm)	Mean (mm)	SD	SE
10	10	3.37-3.79	3.63	0.13	0.04
12	10	3.31-4.00	3.62	0.21	0.07
14	10	3.63-4.10	3.93	0.18	0.06
17	10	3.63-4.00	3.81	0.15	0.05
18	10	3.63-4.10	3.94	0.16	0.05
20	10	3.52-4.16	3.82	0.18	0.06
24	10	3.52-4.00	3.71	0.14	0.04
26	10	3.42-3.84	3.56	0.13	0.04

significantly smaller than the maximum response at 18°C (Least Significant Difference, Sokal and Rohlf 1969). It seems reasonable to assume that larvae reared at temperatures of 12°C or lower and 24°C or higher converted yolk into body tissue at suboptimal levels. Analysis of variance showed that maximum mean lengths attained by larvae reared at 14°, 17°, 18°, and 20°C were not significantly different ( $P > 0.05$ ).

## DISCUSSION

Figure 10 shows a graphic summary of various field and laboratory-deduced temperature ranges and limits for the distribution and survival of *E. mordax*. A temperature range of about 4.5°C lies between the highest temperatures that anchovy adults have been found in nature (25°C, Baxter 1967) and the experimentally determined upper lethal temperature for juveniles and adults (29.5°C). Anchovy had been maintained in the laboratory at 28°C for weeks with no apparent ill effects. The fish are extremely active at this temperature and their metabolic requirements are undoubtedly considerable. Anchovy maintained at 28°C and fed the standard ration lost weight. The upper environmental temperature limit and southern distributional limit of *E. mordax* may be dictated by metabolic demands which outweigh the ration supplied by the environment. Maximum temperatures off Cabo San Lucas, which is the southern range limit for *E. mordax*, exceeds 25°C (Lynn 1967). Interestingly, 25°C corresponds to the highest temperature that juvenile *E. mordax* would venture into when tested in laboratory thermal gradients (Brewer 1974). Moreover, the plateau in the thermal tolerance polygon (Figure 3) shows that acclimation temperatures of 24°C and above have little effect on increasing the incipient upper lethal temperature. Apparently the anchovy's overall mechanisms for

physiological compensation begin to break down at temperatures above 25°C.

Reid's (1967) observation that *E. mordax* may overwinter at temperatures of 7° or 8°C off British Columbia is of special interest. These fish may be viable less than 1°C of their lower lethal temperature. Juvenile and adult anchovy acclimated to 8°C in the laboratory and transferred to 7°C made no effort to consume food offered to them after 5 days at the lower temperature. I have not confirmed this by stomach examination, but feeding, if it takes place at all, is minimal at this low temperature.

It is important to consider the possibility that the thermal tolerance and resistance of *E. mordax* may be different for northern, central, and southern populations. Apparently genetically distinct, these populations were first identified on the basis of meristic characters by McHugh (1951) and later on by serum transferrin analysis conducted by Vrooman and Smith (1971). If the thermal requirements of these populations were distinct, I would anticipate their reproductive temperature ranges to vary accordingly. Richardson's (1973) data on anchovy spawning off Oregon discount this. In any case, thermal resistance experiments

on samples from each population would be of interest.

Experiments on the resistance of juvenile and adult anchovy to a high lethal temperature showed no significant difference in the mean resistance times for fish of different sizes or for fish maintained under different photoperiods. However, females were more resistant than males, and animals tested in the morning showed greater resistance than those tested in the evening. Investigators have variously shown significant differences in one or more of the factors tested here, depending on the species. Thermal resistance has been found to vary according to size, with large *Oncorhynchus* (Salmonidae) and *Carassius* (Cypriidae) more resistant to cold (Brett 1952; Hoar 1955, respectively) and large *Clupea* (Clupeidae) less resistant to heat (Brawn 1960). *Carassius* maintained under long photoperiods were more resistant to high temperatures than fish maintained under short photoperiods, while resistance to cold temperatures was greater for the short photoperiod fish (Hoar 1956). Hoar discovered that male *Carassius* were more resistant to low temperature extremes than females. Heath (1963) observed slight differences in critical thermal

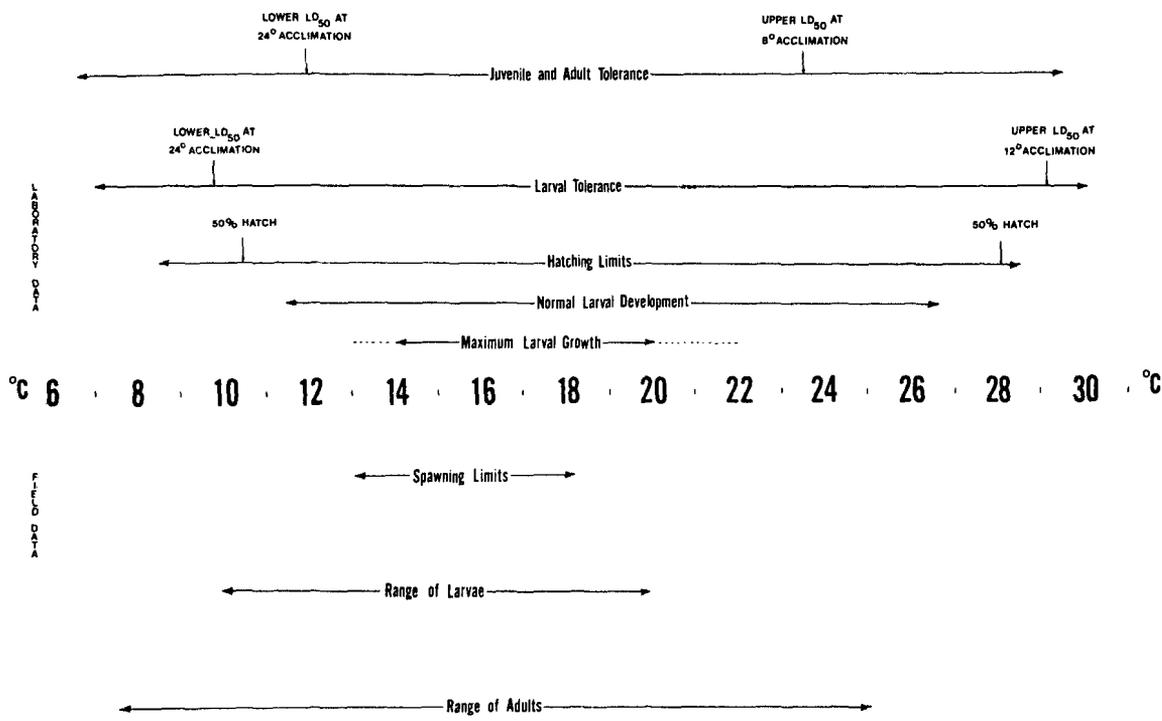


FIGURE 10.—Field and laboratory deduced thermal limits for the distribution and survival of *Engraulis mordax*.

maximum temperatures for *Salvelinus* (Salmonidae), depending on the time the test was conducted. He also noticed that maximum tolerance followed a 24-h cycle and suggested that this was a physiological adaptation to natural habitats with 24-h variations in temperature.

The present experiments on *E. mordax* were conducted in the fall when anchovy presumably ascend from deep water to warm surface waters in the evening (California Department of Fish and Game 1971). If a circadian cycle of thermal resistance existed in anchovy, one might anticipate maximum resistance to high temperatures to occur in the evening. The data in Table 1 suggest that, under laboratory conditions, resistance to high temperature is reduced in the evening.

The embryonic and larval stages of pelagic fishes are potentially the most vulnerable ones to thermal stresses. While juvenile and adult fishes may detect and avoid unfavorable environmental conditions (Bull 1928; Doudoroff 1938; Alabaster and Robertson 1961; Coutant 1969), the eggs and planktonic larvae of fishes such as *E. mordax* are at the mercy of currents which might carry them into environments unfavorable for growth or survival. Reviews by de Sylva (1969) and Brett (1970) have shown that on the average, marine fish larvae are one-third to one-half as tolerant to thermal stresses as their conspecific adults. Normal development of *E. mordax* is inhibited below 11.5°C and above 27.0°C. Larvae held at temperatures below 11.0°C for short periods become inactive, making little effort to avoid capture by pipette.

The survival of pelagic larvae is dependent on the early consumption of prey species and the ability to avoid predators (Lasker et al. 1970). The degree to which these two processes can be accomplished is largely dependent on the optimal development of swimming ability, precise biting reflexes, and visual acuity (Hunter 1972). Since swimming ability is proportional to larval size, the development of maximum growth potential should be of distinct survival value. Maximum growth of larvae in the yolk-sac stage, in turn, is dependent on the efficient utilization of the limited yolk reserve, i.e., its conversion into body tissues.

Growth of anchovy larvae in the yolk-sac stage is maximal in experimental temperatures between 14° and 20°C. Variation within this range may be highly significant but is difficult to test. Although growth rates of anchovy larvae in the yolk-sac stage increase with increasing tempera-

tures, the maximum size attained by the larvae decreased at high temperatures.

Thermal tolerance limits have been determined for anchovy larvae and juveniles and adults by tests that considered the LD<sub>50</sub> as a lethal end point. LD<sub>50</sub> temperatures do not represent "safe" levels and have been used merely because of convention. Any temperature level that produces a lethal response significantly greater than the maximum response at control temperatures should be considered excessive. This would represent the most realistic end point to insure environmental quality. The thermal death of even a few individuals at any particular temperature level suggests that the survivors are under severe stress, leaving them unable to compete successfully for limited resources or avoid predation. For acclimation temperatures of 8°, 12°, 16°, 20°, and 24°C, a range of temperatures encountered by juveniles and adults in nature, immediate exposure to high temperatures less than 23.0°, 24.0°, 25.5°, 26.5°, and 27.5°C, respectively, would be tolerated by fish from southern California without significant mortality from the direct effects of temperature alone. Likewise, for the same acclimation temperatures, juvenile and adult anchovy could tolerate lows of 7.5°, 10.0°, 12.5°, 13.5°, and 14.5°C, respectively. Larvae in the yolk-sac stage can tolerate limited exposure (24 h) to any temperature <28.0°C and >12.0°C. Regardless of acclimation temperature, larvae in the yolk-sac stage, juveniles, and adults can endure sudden temperature increases and decreases between the limits of 14.5° and 23.0°C without significant lethality from direct temperature effects alone.

Although the gross effects of high and low temperature extremes have been quantified, the physiological and biochemical factors that are responsible for thermal death and temperature acclimation are poorly understood. Various mechanisms to account for these phenomena have been discussed by Hochachka and Somero (1971) and Hazel and Prosser (1974). Evidence suggests that qualitatively different enzymes (isoenzymes) may be synthesized during thermal acclimation, and "warm" and "cold" enzyme variants have been described (Hochachka 1967; Hochachka and Somero 1968; Hebb et al. 1969). Enzyme inactivation has been suggested as a cause of thermal death, but it is ". . . undoubtedly more subtle than gross protein denaturation" (Hochachka and Somero 1971:139). The reaction velocities ( $K_m$ ) of enzymes may drop below certain critical levels at

high and low temperatures, resulting in the disruption of basic physiological functions such as osmoregulation, respiration, and overall nervous system integration (Prosser 1973).

It is unlikely that the offshore realm of any ocean could ever be significantly affected by artificial thermal input. Projected energy needs for the decades ahead and their associated requirements for immense volumes of water for cooling (electric power generating) and heating (LNG) may pose a serious environmental threat in near-shore areas, especially bays, harbors, and estuaries. As a case-in-point, juvenile northern anchovy find the confined waters of the Los Angeles-Long Beach Harbor a suitable habitat. Brewer (1975a) found anchovy egg densities as high as 35/m<sup>3</sup> of surface water within 0.5 mile of the harbor breakwater. These areas will be affected by seawater intake pipes, and thermal effluent plumes and *E. mordax* embryos would be highly susceptible to entrainment. Eggs in the blastodisc stage are most sensitive to abrupt changes in temperature. If one considers the high temperature extremes where mortality begins to exceed the control mortality as unsafe, anchovy embryos should not be allowed to remain in temperatures of 35.5°, 30.5°, 30.0°, and 27.5°C for periods longer than 1, 3, 5, and 60 min, respectively. While embryos proved insensitive to the effects of temperatures as low as 0.5°C for 60-min exposures, it is questionable whether these sensitive developmental stages could withstand the turbulence and mechanical shock associated with heat exchange systems or thermal effluent outfalls. In this respect, larvae are most vulnerable, and Lasker (1964) found this vulnerability increased with decreasing temperatures below 14°C for Pacific sardine, *Sardinops*, larvae which are morphologically similar to anchovy larvae. Their thin integument and fragile bodies are easily damaged. Extreme care was taken in the present study when the larvae were transferred from incubation to test jars, but control survival was only 77.5%. Survival of larvae in experiments that did not involve transfer to rearing vessels was over 90%. Serious consideration must therefore be given to the location of intake pipes and effluent discharge to avoid trapping eggs and larvae. These stages are probably too small to be excluded by screening.

Many more experiments are required to understand the dynamics of the thermal requirements of *E. mordax*. It may be unreasonable to assume that

there is one optimal temperature for anchovy well-being. Activity cycles or rhythms (e.g., the evening spawning cycle) may be present in natural populations which require diel temperature changes (e.g., achieved through vertical migration). Temperature optima for reproduction or the growth of larvae in the yolk-sac stage may differ from optima for growth of juveniles and adults which must respond to fluctuating food levels. Brett et al. (1969), experimenting with *Onchorhynchus nerka*, found that as food rations were decreased, temperatures required for maximum growth rates also decreased. When food rations were not limiting, growth rates increased as the temperature increased to a certain optimal level, after which growth rates decreased rapidly.

In conclusion, the potential responses of the northern anchovy to temperature are many and varied. They depend upon the degree and rate of temperature change, length of exposure to a particular temperature, the previous thermal experience of the fish, and the effects of interactions among other environmental variables, both biotic and abiotic. Furthermore, these responses vary with ontogeny.

Although expatriated individuals may temporarily tolerate environmental extremes, the distribution and survival of *E. mordax* are ultimately dependent upon those physicochemical characteristics of the environment conducive to spawning. For the present, such an environment is best described as that part of the California Current where surface water temperatures reach 13°-18°C during at least part of the year (Ahlstrom 1956, 1959, 1966, 1967; Richardson 1973).

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